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Summary and Implications

Pacific white shrimp are of particular economic importance to the global shrimp aquaculture industry. We utilized the limited public sequence information, mainly genetic markers called single nucleotide polymorphisms (SNPs) and expressed sequence tags, to discover markers for the construction of the first SNP genetic map for Pacific white shrimp. In total, 1344 putative SNPs were discovered, and out of 825 SNPs genotyped, 418 SNP markers from 347 contigs were mapped onto 45 sex-averaged linkage groups, with approximate coverage of 2071 and 2130 cM for the female and male maps, respectively. Comparative mapping to model organisms, *Daphnia pulex* and *Drosophila melanogaster*, revealed extensive rearrangement of genome architecture for *L. vannamei*, and that *L. vannamei* was more related to *Daphnia pulex*. This SNP genetic map lays the foundation for future shrimp genomics studies, especially the identification of genetic markers or regions for economically important traits.

Introduction

The construction of a genetic map is the first step to identify genetic markers or genomic regions underlying economically important traits. It then can be used to make breeding schemes using marker-assisted selection (MAS) to improve the performance of farm animals. We have discovered a large number of SNP markers, using a combination of bioinformatics and molecular technologies and tools. Here we report the construction of a genetic map with high density coverage of the whole shrimp genome, which would be of great value for future breeding programs.

Materials and Methods

The resource family was produced using a standard F_2-design. The F1 animals were generated by crossing males from one shrimp line (red) with females from another line (yellow). Crosses between six F1 animals produced three F2 families. A total of 144 animals from three F2 families, eight grandparents and six F1 parents, were used in the construction of the map. Individual tissue was collected from all animals and stored at -80°C. Genomic DNA was extracted using the DNeasy Midi kit (Qiagen). SNPs discovered by direct sequencing of PCR amplicons were genotyped by the Sequenom MassArray iPLEX™ platform. After quality control, the genotyping data were formatted and exported into CRIMAP, for the construction of a genetic linkage map. Linkage groups were numbered according to their lengths.

EST sequences from SNP discovery were used as queries to blast against the Drosophila melanogaster (fruitfly) and Daphnia pulex (water flea) sequence databases (BLASTX, E-value <10)5), because no genome sequence of other closely related decapod was available. We used the first sequenced crustacean genome, the Daphnia genome database (http://wfleabase.org/) and the JGI Genome Portal (http://genome.jgi-psf.org/Dappu1/Dappu1.home.html).

Results and Discussion

The genome of Pacific white shrimp appears to have high rates of duplication, which was indicated by a significant number of paralogous and multiple sequence variations, 7.14% and 3.44% respectively. Also, 11.53% of the markers failed genotyping and 17.91% were homozygous for all animals. In all, 453 SNPs were successfully genotyped and were informative in at least one F2 family, and these were used for the construction of a genetic linkage map. Of all 453 SNPs, 418 were incorporated into the linkage map. These SNPs belonged to 347 contigs, 67 of them having two SNPs genotyped, two having three SNPs genotyped and the remaining 278 having one SNP. The sex-averaged map has 418 markers grouped onto 45 linkage groups, with group lengths ranging from 0 to 171.3 cM and a total coverage of 2262.3 cM. The grandparental genotyping data for the resource families helps in the determination of the marker linkage phase and the precise ordering of genetic markers.

Comparative genomics can provide valuable information about the architecture and functional organization of the genome, especially in species without genome sequences. Our analysis shows that some *L. vannamei* genes are related to their orthologs in the crustacean *Daphnia pulex*, whereas others are more related to orthologs in *Drosophila melanogaster*. Half of the unique ESTs/contigs (172) seem specific to *L. vannamei*, as no hits were found in the two model organisms

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